PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Lieven De Veylder *et al.*	**Examiner:**	Cynthia Collins
Serial Number:	09/574,735	**Art Unit:**	1638
Filed:	May 18, 2000	**Docket:**	1187-2 CIP
For:	CYCLIN-DEPENDENT KINASE INHIBITORS AND USES THEREOF		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Sir,

I, Wim J. F. Van Camp declare as follows

1. I am a Belgian citizen residing at 14 Witbakkerstraat, B-9051 Sint-Denijs-Westrem, Belgium.

2. I graduated from Rijksuniversiteit Gent with a First Class Degree in Zoology in 1988 and from Rijksuniversiteit Gent with a Doctor of Philosophy in 1994. From 1994 to 1998 I held the position of Group Leader in the Laboratory of Genetics of Prof. Dr. Marc Van Montagu. Since 1998, I have been working for CropDesign N.V. in various positions and currently as Director, Technology Management. I am first author or co-author of a number of scientific publications.

3. I am a co- inventor of the subject matter in the above-identified application, (hereinafter referred to as the "APPLICATION") and I am familiar with the contents therein. I have read the Official Action of the United States Patent and Trademark Office dated February 25, 2004.

4. The invention of the present APPLICATION is directed to a method for decreasing or increasing cyclin dependent kinase activity in a plant and to the various phenotypic changes which occur in a plant upon doing so.

5. Page 53 of the present application teaches that previously unrecognised amino acid sequence motifs have been identified in plant cyclin-dependent kinase inhibitors (CKIs or ICKs). As indicated on page 53 of the application, the different identified motifs are summarized in Table 2. The same motifs are graphically represented in Figure 12 (not Figure 1 as page 53 indicates).

6. As indicated in Table 2 of the specification, the conserved motifs, Motif 1, Motif 2, and Motif 3 are found in different plant ICKs. As also indicated in Table 2, a consensus sequence for each motif has been formulated. Thus, the sequences which have been designated "Motif 1" in Table 2 are best characterized by the consensus sequence FX_2KYNFD (the sequence set forth in SEQ ID NO:34). The sequences which have been designated "Motif 2" are best characterized by the consensus sequence [P/L]LXGRYEW (the sequence set forth in SEQ ID NO:35); and the sequences which have been designated "Motif 3" are best characterized by the consensus sequence EXE[D/E]FFX_3E (the sequence set forth in SEQ ID NO:36).

7. It is my considered scientific opinion that one skilled in the art, having the present application in hand and reflecting upon Table 2 , would reasonably understand that the seven different *Arabidopsis* ICKs as well as *Chenopodium* and alfalfa ICKs listed therein, each contain the consensus sequences SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, albeit some of the ICKs have one or more amino acid substitutions when directly compared to a particular consensus sequence. It is my further considered scientific opinion that reliable identification of a consensus sequence within a larger sequence often occurs when the consensus sequence is not a perfect match to the published or known consensus sequence. The fact that a consensus sequence located within a larger sequence, does not perfectly match a known or published consensus sequence does not diminish in any way, such identification.

8. The present application provides a teaching with particularity of the principles described in paragraph 7, with respect to the consensus sequences SEQ ID NOs: 34-39. Specifically, page 54, line 12 to page 55, line 18 provides:

As described herein, overall homology between plant ICKs is very low, i.e., lower than 40% whereas identities are under 30%. This hampers the identification of novel ICK genes in plants. Therefore, the delineation of conserved motifs is of utmost importance to enhance identification of said novel plant ICK genes. Presence or absence of (some of) said motifs enabling structural classification of plant ICKs can possible assist in prediction of ICK function thus preventing undue experimentation. Finally, conserved ICK-motifs as identified in the current invention enable construction of functional recombinant plant ICK proteins such as ICK orthologues, via domain shuffling and/or with novel combinations and/or positions of said motifs in said recombinant ICK proteins. Such recombinant ICK proteins will open more new avenues to modifications of plant growth and/or development.

Accordingly, one embodiment of the invention includes DNA sequences coding for a functional plant ICK or an ortholog thereof, which furthermore comprise:

(a) DNA sequences encoding a peptide with the consensus sequence as given in SEQ ID NO:34 or a peptide that is at least 70% identical thereto; and/or

(b) DNA sequences encoding a peptide with the consensus sequence as given in SEQ ID NO:35 or a peptide that is at least 70% identical thereto; and/or

(c) DNA sequences encoding a peptide with the consensus sequence as given in SEQ ID NO:36 or a peptide that is at least 70% identical thereto; and/or

(d) DNA sequences encoding a peptide with the consensus sequence as given in SEQ ID NO:37 or a peptide that is at least 70% identical thereto; and/or

(e) DNA sequences encoding a peptide with the consensus sequence as given in SEQ ID NO:38 or a peptide that is at least 70% identical thereto; and/or

(f) DNA sequences encoding a peptide with the consensus sequence as given in SEQ ID NO:39 or a peptide that is at least 70% identical thereto.

9. The record is replete with examples of ICKs from different plants, containing the consensus sequences identified in this application being used to decrease cyclin-dependent kinase activity in plants with the resultant phenotypic changes as presently claimed. Indeed, many of the different ICKs listed in Table 2, having either a perfect match to consensus sequences SEQ ID No: 34, SEQ ID NO:35 and SEQ ID NO:36, listed therein, or having one mismatch (substitution) or alternatively characterized as being at least 70% identical to the

consensus sequences SEQ ID No: 34, SEQ ID NO:35 and SEQ ID NO:36, have been submitted to the Examiner as actual exemplifications of the presently claimed invention.

10. Thus for example, ICK1 which is listed in Table 2, has been exemplified in Zhou et al. (2002), submitted as Exhibit B with Applicants' response filed August 9, 2002. ICK2, listed in Table 2, is exemplified in Example 16 of the present application. ICK3, listed in Table 2, is exemplified in paragraph 12 of the declaration filed November 17, 2003. ICK6 (called ICK4 by the authors), is exemplified in Zhou et al. (2002) submitted as Exhibit B with Applicants' response filed August 9, 2002. Exhibits 3 and 7 of the declaration submitted November 17, 2003 also exemplify the present invention using ICK4. Paragraph 12 of the declaration submitted November 17, 2003, also exemplifies ICK4. ICK5, also listed in Table 2 is exemplified in Zhou et al. (2002). ICK6 is exemplified in paragraphs 5, 6, and 7 and corresponding Exhibits 1, 2, and 3 of the declaration filed November 17, 2003. ICK7, listed in Table 2, is exemplified in paragraphs 8, 9, and 10 and in corresponding Exhibits 4, 5, and 6 of the declaration filed November 17, 2003. *Chenopodium*, the last ICK listed in Table 2, is exemplified in Zhou et al. (2002).

11. As still further support for the presently claimed invention, provided below is a description of experiments related to decreased seed size, an embodiment of the invention recited in e.g., claims 30 and 47 of the present application.

12. In a first experiment, a genetic construct was made wherein an *Arabidopsis* ICK7 cDNA was placed under the control of a rice promoter functioning in young expanding plant tissues. The construct was then introduced into rice using *Agrobacterium*-mediated transformation. T1 plants obtained from the seeds of T0 plants were then grown in the greenhouse and evaluated. Five transgenic events (each comprising 10 plants) were compared to their null counterparts for seed related parameters, such as seed yield, number of filled seeds and thousand kernel weight (TKW). TKW is the weight of a thousand kernels and thus reflects the weight and size of single seeds. Statistic analyses were based on a two factor ANOVA (analysis of variance). Exhibit A, attached hereto, provides a table of the results of such an analysis. As Exhibit A reflects, all decreases in seed yield, number of filled seeds and TKW were highly significant. To my knowledge, this is the first showing of an *Arabidopsis* CKI introduced into rice. As reflected in Table 2 of the present application, each of the three consensus sequences present in the amino acid sequence of ICK7 has one

mismatch, or is at least 70% identical to the corresponding consensus sequence listed in the bottom line of Table 2, i.e., SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36.

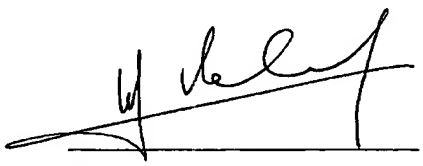
13. In a second experiment, a genetic construct was made wherein a rice (*Oryza sativa*) ICK2 cDNA was placed under the control of a constitutive rice promoter. The construct was then introduced into rice using *Agrobacterium*-mediated transformation. T1 plants obtained from seeds of T0 plants were then grown in a greenhouse and evaluated. Five transgenic events (each event comprising 10 plants) were compared to their null counterparts for seed related parameters, such as seed yield and thousand kernel weight (TKW). As indicated above, TKW is the weight of a thousand kernels and thus reflects the weight and size of single seeds. Again, statistic analyses were based on a two factor ANOVA. The results of the ANOVA are provided at Exhibit B, attached hereto. As Exhibit B reflects, all decreases in seed yield, number of filled seeds and TKW were highly significant.

14. The rice ICK2 cDNA is not listed in Table 2, nor provided by the present application; it was identified after the present application was filed, using the teachings provided therein. Specifically, consensus sequences having at least 70% identity to the amino acid sequences set forth in SEQ ID NOs: 34, 35, and 36 and present in the corresponding rice amino acid sequence were used to identify the rice cDNA as encoding an ICK.

15. The preceding paragraphs and attached exhibits demonstrate that Applicants were in possession of the claimed invention at the time the application was first filed. Applicants have repeatedly demonstrated during prosecution of this application, that CKIs which bind a plant cyclin-dependent kinase having a PSTAIRE cyclin-binding motif, wherein the CKIs comprise the amino acid sequences as set forth in SEQ ID NOs:34, 35, and 36 or else comprise amino acid sequences having at least 70% identities to the amino acid sequences as set forth in SEQ ID NOs:34, 35, and 36, may be used for decreasing or increasing cyclin dependent kinase activity in a plant, thereby obtaining plants with various phenotypes as presently claimed.

16. I declare that all statements made herein of my knowledge are true and that all statements are made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such false statements may jeopardize the validity of the APPLICATION or any patent issuing thereon.

Dated: May 18, 2004



Wim J. F. Van Camp

EXHIBIT A

Expressing an *Arabidopsis* ICK gene in rice

Parameter measured	% Difference	P value
Seed yield	-29%	0.003
Nbr of filled seeds	-26%	0.0048
TKW	-4%	0.0118

EXHIBIT B

Expressing a rice OsICK2 gene in rice

Parameter measured	% Difference	P value
Seed yield	-69%	0.0000
Nbr of filled seeds	-63%	0.0000
TKW	-5%	0.0003